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## **Association of ambient air pollution with age-related macular degeneration and retinal thickness in UK Biobank**

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## **SYNOPSIS**

Age-related macular degeneration (AMD) is the leading cause of vision loss among the elderly in high income countries. Increased exposure to air pollution may be associated with AMD and differences in retinal layer thickness.

## 1 ABSTRACT

2 **Aim:** To examine the associations of air pollution with both self-reported age related  
3 macular degeneration (AMD), and in vivo measures of retinal sub-layer thicknesses.

4 **Methods:** We included 115,954 UK Biobank participants aged 40 to 69 years old in  
5 this cross-sectional study. Ambient air pollution measures included particulate matter,  
6 nitrogen dioxide (NO<sub>2</sub>) and nitrogen oxides (NO<sub>x</sub>). Participants with self-reported  
7 ocular conditions, high refractive error (< -6 or > +6 diopters) and poor spectral-domain  
8 optical coherence tomography (SD-OCT) image were excluded. Self-reported AMD  
9 was used to identify overt disease. Spectral-domain optical coherence tomography  
10 (SD-OCT) imaging derived photoreceptor sub-layer thickness and retinal pigment  
11 epithelium (RPE) layer thickness were used as structural biomarkers of AMD for  
12 52,602 participants. We examined the associations of ambient air pollution with self-  
13 reported AMD and both photoreceptor sub-layers and retinal pigment epithelium  
14 (RPE) layer thicknesses.

15 **Results:** After adjusting for covariates, people who were exposed to higher fine  
16 ambient particulate matter with an aerodynamic diameter <2.5µm (PM<sub>2.5</sub>) (per  
17 interquartile range [IQR] increase) had higher odds of self-reported AMD (OR= 1.08,  
18 p=0.036), thinner photoreceptor synaptic region ( $\beta$ = -0.16µm, p=2.0X10<sup>-5</sup>), thicker  
19 photoreceptor inner segment layer ( $\beta$ = 0.04µm, p=0.001) and thinner RPE ( $\beta$ = -  
20 0.13µm, p=0.002). Higher levels of PM<sub>2.5</sub> absorbance and nitrogen dioxide (NO<sub>2</sub>) were  
21 associated with thicker photoreceptor inner and outer segment layers, and a thinner  
22 RPE layer. Higher levels of PM<sub>10</sub> (PM with an aerodynamic diameter <10µm) was  
23 associated with thicker photoreceptor outer segment and thinner RPE, while higher  
24 exposure to NO<sub>x</sub> was associated with thinner photoreceptor synaptic region.

25    **Conclusion:** Greater exposure to PM<sub>2.5</sub> was associated with self-reported AMD, while  
26    PM<sub>2.5</sub>, PM<sub>2.5</sub> absorbance, PM<sub>10</sub>, NO<sub>2</sub> and NO<sub>x</sub> were all associated with differences in  
27    retinal layer thickness.

## 28 INTRODUCTION

29 Age-related macular degeneration (AMD) is the leading cause of irreversible blindness  
30 in adults 50 years and above in high income countries.<sup>1</sup> Dry AMD is characterized by  
31 progressive dysfunction of the retinal pigment epithelium (RPE), photoreceptor loss  
32 and retinal degeneration..<sup>2</sup> By 2020, the global projected number of people with AMD  
33 is approximately 200 million, increasing to nearly 300 million by 2040.<sup>3</sup> Well-known  
34 risk factors include older age, smoking and genetic factors.<sup>1</sup> A constellation of adverse  
35 factors (both risk genotypes, smoking and body mass index [BMI]  $\geq 25$ ) together  
36 increases the risk 19-fold.<sup>4</sup> As smoking tobacco is a risk factor, it is plausible that  
37 ambient air pollution may also be a modifiable risk factor.

38

39 Air pollution is one of the world's most important environmental health risks. It is  
40 associated with increased mortality and morbidity.<sup>5</sup> Exposure to air pollution is  
41 associated with pulmonary and cardiovascular disease<sup>6</sup> and eye diseases including  
42 glaucoma<sup>7</sup> and AMD.<sup>8</sup> The mechanisms of air-pollution-induced health effects may  
43 likely involve oxidative stress and inflammation.<sup>9</sup> The retina is one of the highest  
44 oxygen-consuming tissues in the human body and resides in an environment that is  
45 primed for the generation of reactive oxygen species (ROS) and resultant oxidative  
46 damage.<sup>10</sup> Oxidative damage increases with age, resulting in retinal dysfunction and  
47 cell loss. Rapid, non-invasive optical coherence tomography (OCT) imaging of the  
48 retina is now commonly used by community opticians and hospital eye clinics and to  
49 assess retinal structural changes associated with AMD, and to guide its  
50 management.<sup>11</sup>

51

If air pollution has an adverse effect on AMD risk, this may offer a new range of interventions for controlling this important condition. We examined data from UK Biobank, a large community-based cohort study. The aim of our study was to evaluate the relationship between ambient air pollution, AMD status and OCT imaging derived structural features of the disease: photoreceptor sub-layer and RPE layer thickness.

## **METHODS**

### **Study population**

UK Biobank (UKBB) is a very large community-based cohort of 502,656 UK residents registered with the National Health Service (NHS) and aged 40–69 years at enrolment. Baseline examinations were carried out between 2006-2010 at 22 study assessment centres. The North West Multi-centre Research Ethics Committee approved the study in accordance with the principles of the Declaration of Helsinki. The overall study protocol (<http://www.ukbiobank.ac.uk/resources/>) and protocols for individual tests (<http://biobank.ctsu.ox.ac.uk/crystal/docs.cgi>) are available online. Participants answered a wide-ranging touch-screen questionnaire covering demographic, socioeconomic, lifestyle, systemic and ocular diseases information. Definition of hypertension was based on self-reported. Physical measures included height and weight. Body mass index (BMI) was defined as weight divided by height squared.

### **Ocular assessment**

Ocular assessment was introduced as an enhancement in 2009 for six assessment centers which are spread across the UK.<sup>12</sup> Habitual visual acuity (VA) was measured

75 using a logarithm of the minimum angle of resolution (LogMAR) chart (Precision  
76 Vision, LaSalle, Illinois, USA) on a computer screen under standard illumination.<sup>12,13</sup>  
77 Refractive error was measured using an autorefractor (Tomey RC 5000, Nagoya,  
78 Japan).<sup>14</sup> High resolution OCT imaging was performed using the Topcon 3D OCT  
79 1000 Mk2 (Topcon Inc, Oakland, NJ, USA) in a dark room, without pupillary dilation  
80 using the 3D macular volume scan (scan settings: 512 horizontal A scans per B scan;  
81 128 B scans in a 6 x 6 mm raster pattern). The Topcon Advanced Boundary  
82 Segmentation (TABS) Algorithm (Version 1.6.1.1)<sup>15</sup> was used to detect retinal layer  
83 boundaries and measure the thickness of the RPE<sup>16</sup> and photoreceptor sub-layers.  
84 (**Supplementary Figure 1**). The TABS segmentation algorithm has been validated  
85 previously showing a high degree of precision and reproducibility compared to manual  
86 segmentation methods.<sup>15</sup> Strict quality control was implemented to exclude images of  
87 poor quality as described in detail previously.<sup>17</sup> OCT scans with image quality score  
88 (signal strength) < 45 were excluded. Several segmentation indicators were calculated  
89 to identify poor scan quality or segmentation failures. Participants with the poorest  
90 20% of images for each of these indicators were also excluded. These indicators  
91 included an inner limiting membrane (ILM) indicator, a validity count, and motion  
92 indicators. The ILM indicator was a measure of the minimum localized edge strength  
93 around the ILM boundary across the entire scan. It is useful for identifying blinks, scans  
94 that contain regions of severe signal fading, and segmentation errors. The validity  
95 count indicator is used to identify scans with a significant degree of clipping in the OCT  
96 scan's z-axis dimension. The motion indicators use both the nerve fibre layer and the  
97 full retinal thicknesses, from which Pearson correlations and absolute differences  
98 between the thickness data from each set of consecutive B-scans are calculated. The  
99 lowest correlation and the highest absolute difference in a scan serve as the resulting



indicator scores and identify blinks, eye motion artifacts, and segmentation failures. The image quality score and the aforementioned indicators usually are highly correlated.<sup>18</sup>

### **Definition of AMD status**

Definition of AMD status was based on self-reported data. AMD status was determined as those who selected “macular degeneration” from a predefined list of eye disorders to the question “Has a doctor ever told you that you have any of the following problems with your eyes?” We also carried out a validation of self-reported AMD status by carrying out masked grading of the retinal OCT and fundus images for features of AMD based on the Beckman AMD classification on a random subset of age-matched participants.<sup>19</sup>

### **Estimates of air Pollution**

The air pollution estimates were provided by the Small Area Health Statistics Unit (<http://www.sahsu.org/>) as part of the BioSHaRE-EU Environmental Determinants of Health Project (<http://www.bioshare.eu/>), and were linked centrally to the assessment data by UK Biobank analysts (<http://biobank.ctsu.ox.ac.uk/crystal/docs/EnviroExposEst.pdf>). Detailed estimates of air pollution parameters have been published.<sup>20</sup> The annual average concentration of PM<sub>2.5</sub> (aerodynamic diameter of less than 2.5µm), PM<sub>coarse</sub> (aerodynamic diameter between 2.5 and 10µm, PM<sub>10</sub> (aerodynamic diameter of less than 10µm), PM<sub>2.5</sub> absorbance (a measurement of the blackness of PM<sub>2.5</sub> filter – a proxy for elemental or black carbon), nitrogen dioxide (NO<sub>2</sub>) and nitrogen oxides (NO<sub>x</sub>) were calculated centrally by the UK Biobank using a land use regression model developed by the

European Study of Cohorts for Air Pollution Effects (ESCAPE) project (<http://www.escapeproject.eu/>).<sup>21</sup> By using the predictor variables obtained from the Geographic Information System such as traffic, land use, and topography, the land use regression models calculate the spatial variation of annual average air pollution concentration at participants' residential addresses given at baseline visit. NO<sub>2</sub> annual concentration data were available for four years (2005, 2006, 2007 and 2010), while PM<sub>10</sub> data was available for 2007 and 2010. We averaged the values to obtain the mean estimate. All other particulate matter and nitrogen pollutants had the exposure data for a single year (2010).

#### **Inclusion and exclusion criteria**

A uniform set of exclusion criteria was applied in analysis of AMD status, photoreceptor layer and RPE thickness (**Figure 1**). We excluded data from: (1) participants who withdrew consent; or (2) had self-reported diabetes-related eye disease, eye injury resulting in vision loss or other serious eye conditions; high refractive error (< -6 diopters [D] or > +6D) or (3) participants who had poor OCT image scans using TABS software.<sup>16,22</sup> These participants were excluded because of the well-recognized impact these factors have on retinal layer thickness.<sup>23</sup>

#### **Statistical analysis**

The present analysis was based on cross-sectional data collected at one point in time. For this analysis, if both eyes of a patient were eligible for inclusion in the analysis, one eye was randomly selected using STATA software (version 13, StataCorp LP, College Station, TX, USA). We examined the baseline characteristics of participants

included for each specific outcome (self-reported AMD and retinal layers). Descriptive statistics for continuous variables are presented as mean (standard deviation [SD]), whereas categorical variables are presented as number (percentage). We examined the associations of each air pollutant (independent variables) with self-reported AMD (dependent variable) using logistic multivariable regression models, adjusted for age, sex, race, Townsend deprivation index, BMI, smoking status, and refractive error. The associations of air pollutants with photoreceptor sub-layers and RPE thicknesses (dependent variables) were adjusted for the same variables, using linear multivariable regression models. The effect estimates represent the change in self-reported AMD and retinal layers variables per interquartile range (IQR) increment in air pollution. Statistical significance was set at  $p < 0.05$  for the outcomes self-reported AMD and RPE thickness. When photoreceptor sub-layer thickness was analyzed as an outcome, statistical significance was set at  $p < 0.002$  after Bonferroni correction as we examined six different types of air pollutants with four distinct photoreceptor related layers. In sensitivity analysis, we examined the associations of air pollutants with visually significant self-reported AMD. Visually significant self-reported AMD was defined as self-reported AMD participants with VA worse than LogMAR 0.3 (equivalent to Snellen 20/40), while non-visually significant self-reported AMD was defined as those with VA of LogMAR 0.3 or better.

## Results

Of the 133,964 participants who completed ocular assessment, 24 participants withdrew their consent. Of the 133,940, we excluded 13,329 participants according to the exclusion criteria (**Figure 1**), leaving data on 120,611 participants. There were

complete data (age, sex, race, Townsend deprivation index, BMI, smoking status, refractive error, self-reported AMD and air pollution measures) for 115,954 participants. Of the 115,954, there was complete OCT imaging data on retinal layers for 68,088 participants. We excluded 15,486 participants according to the exclusion criteria for OCT. Hence, 52,062 participants were included in the analysis for examining RPE and photoreceptor layer thickness. This large number of exclusions for retinal layers was because of a later start for OCT imaging in UK Biobank, meaning a smaller number of people were scanned.

The characteristics of participants with data on self-reported AMD and a sub-group with data on retinal layer are shown in **Table 1**. Both groups had similar sociodemographic and clinical characteristics. Compared to participants with self-reported AMD, those without self-reported AMD were more likely non-white (9.1% vs 7.0%;  $p=0.01$ ), younger (56.8 years vs 61.6 years), more likely male (46.0% vs 40.9%), more likely to come from a more deprived area (less negative Townsend deprivation index) (-1.1 vs -1.4) and more likely to be smokers (9.7% vs 7.6%) (all  $p<0.001$ ) (**Supplementary Table 1**). The distribution of ambient air pollution exposure of participants with data on self-reported AMD and a sub-group with retinal layer data are shown in **Supplementary Table 2**. The mean [SD] of the various retinal layers are as follows: total length of photoreceptor (142.1 $\mu$ m [8.2 $\mu$ m]), photoreceptor synaptic region (80.4 $\mu$ m [6.6 $\mu$ m]), photoreceptor inner segment (23.8 $\mu$ m [2.0 $\mu$ m]), photoreceptor outer segment (37.9 $\mu$ m [4.3 $\mu$ m]) and RPE (25.6 $\mu$ m [7.2 $\mu$ m]). Of the 115,954 participants, 1,286 (1.1%) were diagnosed with AMD. Masked grading of OCT and retinal fundus images from 119 participants (60 with self-reported AMD and 59 without self-reported AMD) showed that 75% of those with self-reported AMD had

OCT features of AMD while only 12% of those without self-reported AMD had OCT features of AMD.

Participants exposed to higher levels of PM<sub>2.5</sub> concentration were 8% more likely to have self-reported AMD (OR 1.08, 95% CI 1.01 to 1.16; p=0.036, per IQR increase) (**Table 2**). Following Bonferroni correction, higher levels of PM<sub>2.5</sub> and NO<sub>x</sub> were associated with thinner photoreceptor synaptic region (**Table 3**). In contrast, per IQR increase in PM<sub>2.5</sub>, PM<sub>2.5</sub> absorbance and NO<sub>2</sub> were associated with a thicker photoreceptor inner segment layer. Exposure to higher levels of PM<sub>2.5</sub> absorbance, PM<sub>10</sub> and NO<sub>2</sub> were associated with a thicker photoreceptor outer segment layer (**Table 3**). Higher concentration of PM<sub>2.5</sub>, PM<sub>2.5</sub> absorbance, PM<sub>10</sub> and NO<sub>2</sub> were associated with a thinner RPE layer (**Table 4**). In addition, we examined the association of smoking status with self-reported AMD. Among participants with self-reported AMD, 510/1,286 (39.7%) and 101/1,286 (7.9%) were previous and current smokers, respectively. After adjusting for age, sex, race, Townsend deprivation index, BMI, SER and PM<sub>2.5</sub>, compared to never smoking, previous and current smokers were not associated with self-reported AMD (p>0.05). We have additionally adjusted for hypertension in the multivariable models in view of its relationship with AMD<sup>24</sup> and air pollution.<sup>25</sup> The associations of air pollutants with self-reported AMD, photoreceptor sub-layers and RPE thickness did not differ after additional adjustment for hypertension. Sensitivity analysis showed that participants with higher exposure to PM<sub>2.5</sub> was marginally associated with visually significant self-reported AMD (n=167) (OR 1.18, 95% CI 0.98 to 1.41; p=0.08, per IQR increase) compared to participants with either no self-reported AMD or those with non-visually significant self-reported AMD, although it was not statistically significant. None of the other air pollutants were

statistically significant with visually significant self-reported AMD. In the sensitivity analysis, we have also additionally adjusted for smoking pack years and there was a borderline significant association between  $PM_{2.5}$  and self-reported AMD (OR 1.07, 95% CI 0.99 to 1.16;  $p=0.07$ , per IQR increase).

## Discussion

In this large study of UK Biobank participants, we have identified novel associations between ambient outdoor air pollutant levels at participants' residential addresses with self-reported AMD, and also with retinal structure (including thickness of photoreceptor and RPE layers on OCT imaging).

Our results showed that greater ambient  $PM_{2.5}$  exposure was associated with increased odds of AMD and corresponding retinal thicknesses (specifically photoreceptor sub-layer and RPE). No such significant associations were observed for  $PM_{coarse}$ . This may be explained by differences in the sites of deposition in the respiratory tract and the sources and chemical composition for these different-sized PM.<sup>26</sup>  $PM_{coarse}$  are primarily produced from mechanical grinding, windblown dust, and agricultural activities, and mainly deposit in the upper and larger airways. In contrast,  $PM_{2.5}$  particles are mainly from combustion process and are able to reach the smaller airways and alveoli and are transmitted to the blood,<sup>27</sup> causing a cascade of physiological events associated with morbidity and mortality.<sup>5,28</sup> The deeper penetration of  $PM_{2.5}$  may account for the stronger associations of  $PM_{2.5}$  with self-reported AMD and structural biomarkers observed in our study.

246 NO<sub>2</sub> is a product of combustion, primarily from traffic- and industrial sources, and one  
247 of the most notable ambient air pollutants associated with health effects.<sup>29,30</sup> Similarly,  
248 NO<sub>x</sub> is produced from the reaction of nitrogen and oxygen gases in the air during  
249 combustion.<sup>31</sup> NO<sub>x</sub> contributes to the formation of fine particles and ground level  
250 ozone. PM<sub>2.5</sub> absorbance, a measurement of the blackness of PM<sub>2.5</sub> filter – a proxy  
251 for elemental or black carbon, is also an indicator of combustion particles. Since the  
252 major source of NO<sub>2</sub>, NO<sub>x</sub> and PM<sub>2.5</sub> absorbance is from combustion particles, it may  
253 explain the similar associations observed between these air pollutants with the retinal  
254 structures. A recent longitudinal population-based study using data from the Taiwan  
255 National Health Insurance Program between years 2000-2010 included 39,819 AMD-  
256 free participants, with 1442 participants developing AMD during the 11-year follow up.  
257 AMD status was defined via International Classification of Diseases, Ninth Revision,  
258 Clinical Modification (ICD-9-CM). Compared to participants in the lowest exposure  
259 quartile, those in the highest quartile of NO<sub>2</sub> and carbon monoxide (CO) had increased  
260 risk of self-reported AMD (NO<sub>2</sub>: HR=1.91, 95% CI 1.64-2.23, p<0.001 and CO:  
261 HR=1.84, 95% CI 1.50-2.15, p<0.001, respectively).<sup>8</sup> The difference in findings  
262 between ours and the Taiwanese study may be related to the study population,  
263 definition and proportion of AMD cases, type and method of estimating the exposure  
264 of air pollutants and type of covariates adjusted in the multivariable models. Compared  
265 to our study, the Taiwan study included slightly older participants (mean= 62 years vs  
266 56 years), had a slightly higher proportion of AMD (3.6% vs 1.1%) and estimated a  
267 smaller number of air pollutants (two air pollutants including NO<sub>2</sub> and CO vs six air  
268 pollutants). In addition, the participant's living area was defined based on the treatment  
269 venue for acute upper respiratory tract infection in the Taiwan study. The effect of

pollution on retinal structure associated with AMD were not examined in the Taiwan study.

Ambient air pollution could plausibly be associated with AMD through oxidative stress or inflammation. Oxidative damage induces many adverse biological effects including lipid, protein, deoxyribonucleic acid (DNA) oxidation, initiation of proinflammatory processes,<sup>28</sup> and RPE apoptosis.<sup>32</sup> Atrophic or “dry” AMD, also known as geographic atrophy is by degeneration of RPE cells, followed by loss of photoreceptor cells and choriocapillaris.<sup>33</sup> Since the RPE is involved in the turnover of photoreceptor outer segments, RPE dysfunction may lead to thickening of photoreceptor outer segments.

Our results showed that PM<sub>2.5</sub> and NO<sub>x</sub> were associated with a thinner photoreceptor synaptic region. This is in agreement with a reduction in the number of photoreceptor synaptic terminals overlying drusen in AMD.<sup>34</sup> In contrast, PM<sub>2.5</sub>, PM<sub>2.5</sub> absorbance and NO<sub>2</sub> were associated with thicker photoreceptor inner segment, while PM<sub>2.5</sub> absorbance, NO<sub>2</sub> and PM<sub>10</sub> were associated with thicker photoreceptor outer segment. As mitochondria are prominent in photoreceptor inner segments, oxidative stress may induce mitochondrial swelling,<sup>35</sup> leading to a slight thickening in the photoreceptor inner segment. Abnormalities in the photoreceptor inner and outer segments have also been reported in retinal toxicity associated with hydroxychloroquine.<sup>36</sup> Our study did not show an association between air pollution and average total photoreceptor layer thickness, which may be explained by thinning of the synaptic region cancelling out the thickening of the inner/outer segments. In a study by Schuman *et al.*, although the authors reported decreased photoreceptor thickness over drusen, there was a lack of



widespread photoreceptor loss.<sup>37</sup> Hence, it is possible that there was focal loss of the photoreceptor thickness in our study but an overall loss of photoreceptor layer was not observed.

Cigarette smoking may also contribute to particulate matter air pollution.<sup>38</sup> Because of the previously recorded, very strong link between AMD and smoking,<sup>39</sup> and the plausible link between smoking and particulate air pollution, we examined the association between smoking status of participants with self-reported AMD and did not observe a significant association. This suggests that the relationship between PM<sub>2.5</sub> and self-reported AMD is not mediated by cigarette smoke. The prevalence of late AMD standardized to the UK population aged 50 years or more and 65 years or more was 2.4% and 4.8%, respectively. Prevalence of geographic atrophy was 1.3% and 2.5% for the respective age groups.<sup>40</sup> The European Eye Epidemiology (E3) Consortium performed a meta-analysis and showed that overall prevalence was 13.2% for early AMD and 3.0% for late AMD for people aged 70 years or older.<sup>41</sup> Compared to the E3 Consortium, participants in UK Biobank are slightly younger and include a healthier population than the rest of UK population.<sup>42</sup> The self-reported AMD cases in our study may represent AMD in the early stages. We compared the visual acuity between participants with and without self-reported AMD. Among those with self-reported AMD, there was a higher proportion of participants with visual impairment (VA worse than LogMAR 0.3) compared to those without visual impairment (1.8% vs 1.0%;  $p < 0.001$ ). The proportion of self-reported AMD (1.1%) in our study may have been underestimated and it is likely that the risk estimates may have been underestimated.

318

319 In addition to the increased risk of AMD associated with higher exposure to air pollution  
320 in the Taiwanese study, other studies in the UK Biobank<sup>43</sup> and China<sup>7</sup> have reported  
321 increased odds of glaucoma with higher exposure to PM<sub>2.5</sub>. In the UK Biobank study  
322 of 111,370 participants, greater exposure to PM<sub>2.5</sub> was associated with both self-  
323 reported glaucoma and retinal structures associated with the disease.<sup>43</sup> Wang *et al.*  
324 reported that higher average levels of PM<sub>2.5</sub> was associated with higher burden of  
325 glaucoma disability, using national level data.<sup>7</sup> The New England-based Normative  
326 Aging Study showed an association between black carbon exposure with IOP that was  
327 greater in individuals with a high oxidative stress allelic score.<sup>44</sup> Taken together, our  
328 results support published findings of increased risk of eye diseases or association with  
329 retinal structures in participants with higher exposure to ambient air pollution. As  
330 certain groups of individuals including people with diabetes mellitus<sup>45</sup> or  
331 hypertension<sup>24</sup> may have increased risk of AMD, it will be useful to explore if these  
332 groups of individuals are at greater risk of eye disease when exposed to air pollution  
333 in future analysis.

334

335 Strength of this study include its large sample size and the highly accurate and  
336 reproducible measurements of the OCT retinal thickness. Limitations of the study  
337 include the UK Biobank is a volunteer cohort, and participants are likely healthier than  
338 the general population. Outdoor air pollution was estimated using the participants'  
339 home address and do not explain all variation in indoor concentrations. As most  
340 individuals spend a large amount of time indoors, individual exposure to all forms of  
341 air pollution may differ from that indicated by the ambient outdoor figures. This is most

likely to be non-differential between cases and controls and will therefore skew the associations towards the null. Another limitation of this analysis was the use of self-report as the sole determinant of AMD status rather than incorporating a qualitative analysis of the colour fundus photographs and SD-OCT imaging, though we did carry out masked grading of retinal imaging in a proportion of participants. This may result in non-differential misclassification bias and most likely bias the estimates towards the null. Although we applied strict automated quality control criteria including a manual check of SD-OCT scans with high and low outlying layer thickness,<sup>17</sup> it was not practical to manually check all OCT scans for segmentation accuracy. Selection bias may exist: out of the 115,954 participants with data on self-reported AMD, 52,602 participants had measurements on outer retinal layers. However, the baseline characteristics (Table 1) across the two AMD-associated outcome groups appear to be similar. The cross-sectional design of our study limits the ability to determine the causality between ambient air pollution and AMD-associated outcomes. Further research is needed to probe the relationship between prior air pollution exposure and risk of incident disease.

In this large study of an older middle-aged UK population, higher PM<sub>2.5</sub> exposure was associated with a higher risk of self-reported AMD, while all pollutants except PM<sub>coarse</sub> were associated with changes in retinal structure (in either photoreceptor sublayer and/or RPE layer thickness). Overall, our findings suggest that ambient air pollution, especially fine PM or those of combustion-related particles, may affect AMD risk. It is possible that the structural features observed may be unrelated to AMD, but associated with pollution induced retinal toxicity. However, the direction of the relationships between air pollution and both AMD and associated retinal layer

367 thicknesses indicate higher exposure to air pollution may make the cells more  
368 vulnerable and increase the risk of AMD. Our findings add to the growing evidence of  
369 the damaging effects of ambient air pollution, even in the setting of relative low  
370 exposure of ambient air pollution. As UK Biobank is a very large prospective cohort,  
371 we anticipate being able to explore the effect of particulate matter on future risk of  
372 AMD. Further studies examining both outdoor and indoor ambient air pollution  
373 estimates on AMD and outer retinal structures may help to substantiate our findings  
374 and understand the implications for retinal disease associated with ageing. If our  
375 findings are replicated, this would support the view that air pollution is an important  
376 modifiable risk factor for AMD.

**Table 1. Demographic, systemic and ocular characteristics of participants with availability of data on self-reported AMD and retinal layers.**

	Participants with data on self-reported AMD (N=115,954)	Participants with data on retinal layers (N=52,602)
<b>Sociodemographic factors</b>		
Age	56.8 (8.0)	56.4 (8.1)
Sex		
Men	53,218 (46%)	24,753 (47%)
Women	62,736 (54%)	27,849 (53%)
Race		
White	105,465 (91%)	48,475 (92%)
Non-white	10,489 (9%)	4,127 (8%)
Townsend deprivation index	-1.1 (3.0)	-1.2 (2.9)
<b>Clinical factors</b>		
Body mass index (kg/m <sup>2</sup> )	27.3 (4.5)	27.2 (4.4)
Smoking status		
Never	64,554 (56%)	29,238 (56%)
Previous	40,224 (35%)	18,421 (35%)
Current	11,176 (10%)	4,943 (9%)
Spherical equivalent (diopters)	-0.1 (2.1)	0.0 (2.0)

Numbers are mean (SD) or no. (%), unless otherwise stated.

AMD= Age-related macular degeneration, PM<sub>2.5</sub>= Particulate matter (aerodynamic diameter of less than 2.5µm), PM<sub>2.5</sub> absorbance= Particulate matter (a measurement of the blackness of PM<sub>2.5</sub> filter – a proxy for elemental or black carbon), PM<sub>coarse</sub> = Particulate matter (aerodynamic diameter between 2.5 and 10µm, PM<sub>10</sub>= Particulate matter (aerodynamic diameter of less than 10µm), NO<sub>2</sub>= Nitrogen dioxide, NO<sub>x</sub>= Nitrogen oxide

**Table 2: Association of ambient air pollution with self-reported age-related macular degeneration (AMD)**

	Multivariate regression		
	OR	(95% CI)	P-value
<b>Air pollution factors</b>			
PM <sub>2.5</sub> (µg/m <sup>3</sup> )	1.08	(1.01, 1.16)	<b>0.036</b>
PM <sub>2.5</sub> absorbance (µg/m <sup>3</sup> )	1.00	(0.93, 1.07)	0.95
PM <sub>2.5-10</sub> (µg/m <sup>3</sup> )	1.01	(0.96, 1.07)	0.58
PM <sub>10</sub> (µg/m <sup>3</sup> )	0.94	(0.86, 1.02)	0.11
NO <sub>2</sub> (µg/m <sup>3</sup> )	0.99	(0.91, 1.08)	0.80
NOX (µg/m <sup>3</sup> )	1.03	(0.97, 1.09)	0.34

The odds ratio represents per IQR increase in exposure variable.

Values are adjusted for age, sex, race, Townsend deprivation index, body mass index, smoking status and spherical equivalent refraction

**Table 3: Association of ambient air pollution with thickness of the photoreceptor sub-layers**

	Multivariate regression											
	Total photoreceptor			Photoreceptor synaptic region			Photoreceptor inner segment			Photoreceptor outer segment		
	$\beta$	(95% CI)	P-value	$\beta$	(95% CI)	P-value	$\beta$	(95% CI)	P-value	$\beta$	(95% CI)	P-value
<b>Air pollution factors</b>												
PM <sub>2.5</sub> ( $\mu\text{g}/\text{m}^3$ )	-0.07	(-0.16, 0.02)	0.15	-0.16	(-0.23, -0.09)	<b>2.0 X 10<sup>-5</sup></b>	0.04	(0.02, 0.06)	<b>0.001</b>	0.05	(0.003, 0.10)	0.04
PM <sub>2.5</sub> absorbance ( $\mu\text{g}/\text{m}^3$ )	0.06	(-0.03, 0.14)	0.22	-0.10	(-0.17, -0.03)	0.004	0.04	(0.02, 0.06)	<b>2.0 X 10<sup>-4</sup></b>	0.12	(0.07, 0.17)	<b>8.7 X 10<sup>-7</sup></b>
PM <sub>coarse</sub> ( $\mu\text{g}/\text{m}^3$ )	-0.04	(-0.11, 0.02)	0.18	-0.03	(-0.08, 0.02)	0.21	-0.008	(-0.02, 0.007)	0.32	-0.003	(-0.04, 0.03)	0.85
PM <sub>10</sub> ( $\mu\text{g}/\text{m}^3$ )	0.04	(-0.06, 0.14)	0.47	-0.05	(-0.13, 0.03)	0.24	-0.002	(-0.01, 0.007)	0.63	0.09	(0.04, 0.15)	<b>0.001</b>
NO <sub>2</sub> ( $\mu\text{g}/\text{m}^3$ )	0.15	(0.04, 0.26)	0.004	-0.06	(-0.14, 0.03)	0.19	0.04	(0.02, 0.07)	<b>0.001</b>	0.17	(0.11, 0.22)	<b>1.1 X 10<sup>-8</sup></b>
NO <sub>x</sub> ( $\mu\text{g}/\text{m}^3$ )	-0.02	(-0.09, 0.06)	0.63	-0.10	(-0.16, -0.04)	<b>0.001</b>	0.03	(0.008, 0.04)	0.004	0.05	(0.01, 0.09)	0.009

The beta coefficients represent per IQR increase in exposure variable.

Values are adjusted for age, sex, race, Townsend deprivation index, body mass index, smoking status and refractive error.

Statistical significance was set at  $p < 0.002$  after Bonferroni correction.

PM<sub>2.5</sub>= PM<2.5 $\mu\text{g}/\text{m}^3$ ; PM<sub>2.5</sub> ab= (PM<sub>2.5</sub> absorbance) a measurement of the blackness of PM<sub>2.5</sub> filter - a proxy for elemental or black carbon; PM<sub>coarse</sub>= PM between 2.5 and 10 $\mu\text{g}/\text{m}^3$ ; PM<sub>10</sub>= PM <10 $\mu\text{g}/\text{m}^3$ ; NO<sub>2</sub>= Nitrogen dioxide; NO<sub>x</sub>= Nitrogen oxide

**Table 4: Association of ambient air pollution with thickness of the retinal pigment epithelium layer**

	Multivariate regression		
	$\beta$	RPE (95% CI)	P-value
<b>Air pollution factors</b>			
PM <sub>2.5</sub> ( $\mu\text{g}/\text{m}^3$ )	-0.13	(-0.21, -0.05)	<b>0.002</b>
PM <sub>2.5</sub> absorbance ( $\mu\text{g}/\text{m}^3$ )	-0.09	(-0.17, -0.008)	<b>0.03</b>
PM <sub>coarse</sub> ( $\mu\text{g}/\text{m}^3$ )	-0.02	(-0.08, 0.04)	0.50
PM <sub>10</sub> ( $\mu\text{g}/\text{m}^3$ )	-0.12	(-0.21, -0.02)	<b>0.01</b>
NO <sub>2</sub> ( $\mu\text{g}/\text{m}^3$ )	-0.12	(-0.21, -0.02)	<b>0.01</b>
NO <sub>x</sub> ( $\mu\text{g}/\text{m}^3$ )	-0.05	(-0.12, 0.02)	0.17

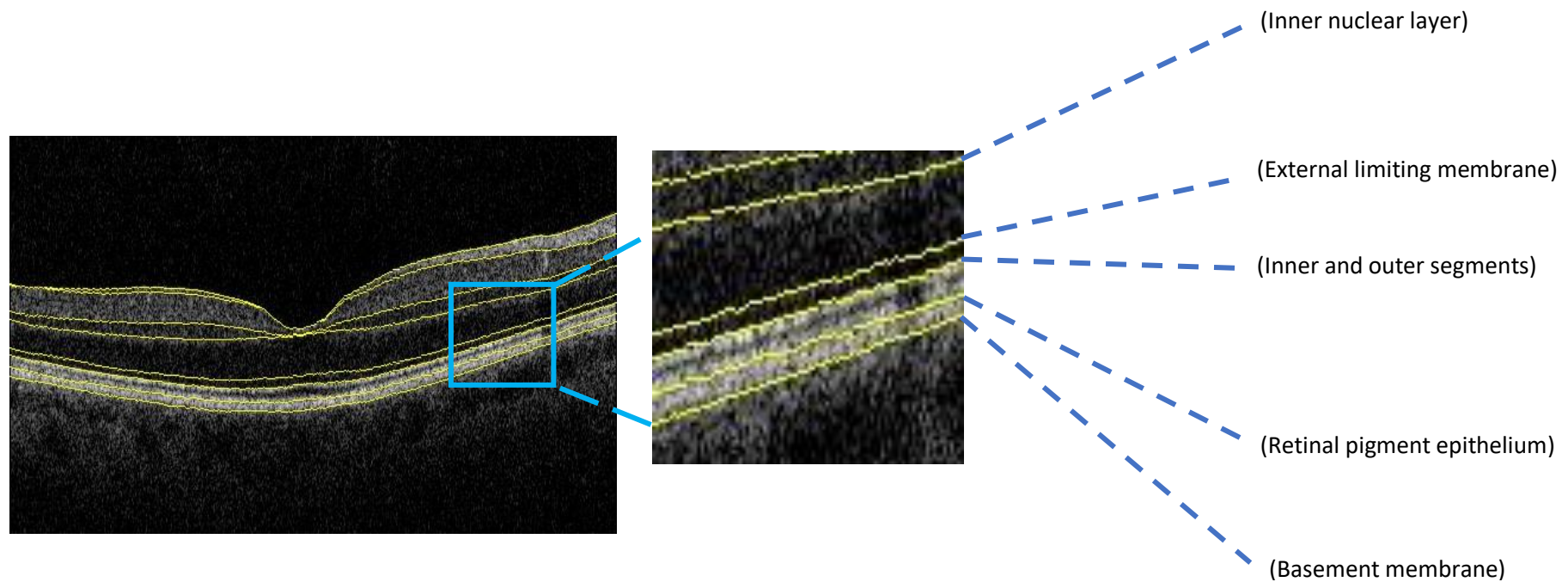
The beta coefficients represent per IQR increase in exposure variable.

Values are adjusted for age, sex, race, Townsend deprivation index, body mass index, smoking status and refractive error.

Statistical significance was set at  $p < 0.05$ .

RPE= Retinal pigment epithelium; PM<sub>2.5</sub>= Particulate matter less than 2.5  $\mu\text{m}$  in aerodynamic diameter; PM<sub>2.5</sub> ab= (PM<sub>2.5</sub> absorbance) a measurement of the blackness of PM<sub>2.5</sub> filter - a proxy for elemental or black carbon; PM<sub>coarse</sub>= Particulate matter between 2.5  $\mu\text{m}$  to 10  $\mu\text{m}$  in aerodynamic diameter; PM<sub>10</sub>= Particulate matter less than 10  $\mu\text{m}$  in aerodynamic diameter; NO<sub>2</sub>= Nitrogen dioxide; NO<sub>x</sub>= Nitrogen oxide





**Supplementary Figure 1.** Spectral-domain optical coherence tomography images with schematic showing representative of total photoreceptor (Inner nuclear layer–Retinal pigment epithelium); photoreceptor synaptic region (Inner nuclear layer- External limiting membrane); photoreceptor inner segment (External limiting membrane-Inner and outer segments); photoreceptor outer segment (Inner and outer segments-Retinal pigment epithelium) and retinal pigment epithelium (Retinal pigment epithelium-Basement membrane).

**Supplementary Table 1. Comparison of characteristics between participants with self-reported AMD and without self-reported AMD**

	<b>No self-reported AMD (N=114,668)</b>	<b>Self-reported AMD (N=1,286)</b>	<b>P-value</b>
<b>Sociodemographic factors</b>			
<b>Age</b>	56.8 (8.1)	61.6 (5.9)	<0.001
<b>Sex</b>			
Men	52,692 (46.0%)	526 (40.9%)	
Women	61,976 (54.0%)	760 (59.1%)	<0.001
<b>Race</b>			
White	104,269 (90.9%)	1,196 (93.0%)	
Non-white	10,399 (9.1%)	90 (7.0%)	0.01
<b>Townsend deprivation index</b>	-1.1 (3.0)	-1.5 (2.9)	<0.001
<b>Clinical factors</b>			
<b>Body mass index (kg/m<sup>2</sup>)</b>	27.2 (4.5)	27.4 (4.3)	0.18
<b>Smoking status</b>			
Never	63,879 (55.7%)	675 (52.5%)	
Previous	39,714 (34.6%)	510 (39.7%)	
Current	11,075 (9.7%)	101 (7.8%)	<0.001
<b>Spherical equivalent (diopters)</b>	-0.08 (2.1)	-0.03 (2.3)	0.40

AMD status was classified based on self-reporting and hospital episode statistics data (ICD10).

Numbers are mean (SD) for continuous variables and no. (%) for categorical variables.

AMD= Age-related macular degeneration

**Supplementary Table 2. Distribution of PM<sub>2.5</sub>, PM<sub>coarse</sub>, PM<sub>10</sub>, NO<sub>2</sub> and NO<sub>x</sub> of participants with availability of data on self-reported AMD and retinal layers**

	Self-reported AMD (N=115,954)		Retinal layers (N=52,602)	
	Median (IQR)	Range	Median (IQR)	Range
PM <sub>2.5</sub> (µg/m <sup>3</sup> )	9.91 (1.07)	(8.17, 19.69)	9.88 (1.12)	(8.17, 19.69)
PM <sub>2.5</sub> absorbance (µg/m <sup>3</sup> )	1.22 (0.33)	(0.83, 4.05)	1.22 (0.33)	(0.83, 3.71)
PM <sub>coarse</sub> (µg/m <sup>3</sup> )	6.19 (0.75)	(5.57, 12.82)	6.21 (0.77)	(5.57, 11.30)
PM <sub>10</sub> (µg/m <sup>3</sup> )	19.37 (2.67)	(13.04, 29.67)	19.33 (2.77)	(13.38, 29.30)
Nitrogen dioxide (NO <sub>2</sub> ) (µg/m <sup>3</sup> )	31.75 (12.08)	(9.44, 102.75)	31.25 (12.63)	(9.44, 86.65)
Nitrogen oxide (NO <sub>x</sub> ) (µg/m <sup>3</sup> )	43.66 (14.38)	(19.74, 263.96)	43.17 (14.97)	(19.74, 263.96)

AMD = Age-related macular degeneration, IQR = Interquartile range, PM<sub>2.5</sub>= Particulate matter (aerodynamic diameter of less than 2.5µm), PM<sub>2.5</sub> absorbance= Particulate matter (a measurement of the blackness of PM<sub>2.5</sub> filter – a proxy for elemental or black carbon), PM<sub>coarse</sub> = Particulate matter (aerodynamic diameter between 2.5 and 10µm, PM<sub>10</sub>= Particulate matter (aerodynamic diameter of less than 10µm), NO<sub>2</sub>= Nitrogen dioxide, NO<sub>x</sub>= Nitrogen oxide

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### **Conflict of Interest:**

CR reports employment by Topcon Healthcare Solutions, Inc. outside the submitted work. PJF reports personal fees from Allergan, Carl Zeiss, Google/DeepMind and Santen, a grant from Alcon, outside the submitted work; PJP reports grants from Topcon Inc, outside the submitted work.

**Ethical approval:** The North West Multi-center Research Ethics Committee approved the study (reference no., 06/MRE08/65), in accordance with the tenets of the Declaration of Helsinki. Detailed information about the study is available at the UK Biobank web site ([www.ukbiobank.ac.uk](http://www.ukbiobank.ac.uk))

### **Authors' Contributions:**

SYLC had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

PJF and PJP led conception and design of the study.

SYLC, PJF and PJP contributed to the data analyses, data interpretation and wrote the draft of the manuscript.

All authors reviewed the results, read and critically revised the manuscript. All authors approved the final manuscript.

The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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